Anxiolytic Effects of Kan-Mai-Ta-Taso-Tang in Rodents

Ming-Tsuen Hsieh* (謝明村), Shu-Er Yang (楊淑娥),
Weng-Huang Peng (彭文煌) and Chi-Rei Wu (吳啟瑞)
Institute of Chinese Pharmaceutical Sciences, China Medical College, 91, Hsueh Shih Road, Taichung, Taiwan, R.O.C.

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ABSTRACT

Kan-Mai-Ta-Taso-Tang (abbreviate as Km) is a common Chinese prescription used to treat insomnia and mania by traditional physicians. Km exhibited sedative and anticonvulsive effects in modern phammacological studies. We therefore investigated the anxiolytic effects of Km in two-way active avoidance, black/white exploration and elevated plus-maze tests in rodents with diazepam and buspirone employed as positive controls.

Km (0.3 and 1.0 g/kg) decreased the response counts of unconditioned stimulus and intertrial crossing in two-way active avoidance tasks and increased the time spent in the black chamber in the black/white exploration test. Km also increased the time spent in the open arms and entries, but decreased the time spent in the close arms in the elevated plus-maze. Furthermore, Km did not display an analgesic effect at any dose. Km at 0.3 and 1.0 g/kg therefore possessed anxiolytic effects in various animal anxiety models.

Key words: Kan-Mai-Ta-Taso-Tang; Two-way active avoidance; Black/white exploration; Elevated plus-maze; Diazepam; Buspirone.

INTRODUCTION

Benzodiazepines are the most widely prescribed medication for the treatment of anxiety disorders. Although many compounds from this substance class have proven clinical efficacy, they also induce a number of side effects, such as sedation, muscle relaxation, and anterograde amnesia. Some sedation and muscle relaxation may benefit the patient and provide important stimulus properties, but amnesia and excessive sedation or muscle relaxation are most often unwanted.[1] For these reasons, drug development in this field re-

cently has been directed to the search of new anxiolytics without unwanted side effects, especially from Chinese herbs. Our early reports have found that Chinese prescriptions such as Suanzaorentang displayed anxiolytic effects mainly via the adrenergic and serotonergic neuron systems.[2-5] Therefore, some Chinese prescriptions are worth studying and using in this field.

Kan-Mai-Ta-Taso-Tang (abbreviate as Km), described in Chin-Kuei-Yao-Lueh, consists of Triticum seeds, Glycyrrhizae Radix and Zizyphus seeds.[6] Km is a common Chinese prescription used to treat insomnia and mania by traditional physicians.[6] Km is mainly used to cure psychiatry disease, postmenopausal syndromes and depressant in recent clinical use.[7] Modern pharmacological studies have shown that Km displays sedative, anticonvulsive, anti-fatigue and anti-anorexia effects.[8-9] Since many drugs that exhibit sedative properties also have anti-conflict effects, possible anxiolytic effects of Km was investigated using animal models of anxiety.

Animal models of anxiety such as two-way active avoidance, black/white exploration and elevated plus-maze tests have typically been pharmacologically validated on the basis of their relative sensitivity to acutely administered benzodiazepine- or barbiturate-type anxiolytic drugs.[10] The animal models have been used to identify novel anxiolytic compounds, and to study the mechanisms whereby these compounds produce their anxiolytic effects. Therefore, we aimed to evaluate the anxiolytic effects of Km by measuring the exploratory behavior of rodents in two-way active avoidance, black/white exploration and elevated plus-maze tests with diazepam and buspirone employed as positive controls.

MATERIAL AND METHODS

Preparation of Extracts

Km, consisting of Triticum seeds (*Triticum aestivum* L.), Glycyrrhizae Radix (*Glycyrrhiza uralensis* Fischer *et* DC.) and Zizyphus seeds (*Zizyphus jujuba* Miller),[6] was purchased from Hsin-Lung Pharmacy. The individual components of Km were mixed in the ratio of 10:3:2 and extracted with 3 liters distilled water. The solution was boiled for 4 hr and the extract was collected. Finally, the extract was reduced to dryness with a vacuum rotary evaporator. Km extract was dissolved in normal saline solution. 0.03, 0.3 and 1.0 g/kg doses of Km extract were administered orally.

Animals

Male ICR mice (20-25 g), housed in groups of ten and Sprague-Dawley rats (220-250 g), housed in groups of five, were used in experiments. They were kept in conditions of constant temperature (23 ± 1 °C) and controlled lighting on a 12 hr light/dark cycle (light,

08:00 to 20:00) with free access to food and water. The experiments were always carried out from 09:00 to 16:00 and animals were never used more than once. Food was deprived for 18 hr before oral drug administration.

Two-Way Avoidance Test in Rats

Two shuttleboxes (Letica Instruments, Barcelona, Spain) were employed. The session included 10 min of habituation followed by a series of 20 trials of avoidance acquisition. Each trial consisted of 10 s of conditioned stimulus (CS, light: 2000 Hz and tone: 70dB simultaneously), followed by a 1 mA footshock of 30 s as aversive unconditioned stimulus (US). The intertrial interval was 30 s. The number of avoidances (crossings in the presence of CS) and the total escape latencies (mean time elapsing between the CS presentation and the response) were scored.[11] The animals were introduced into the apparatus 30 min after Km administration or 20 min after diazepam[12] (0.1 mg/kg, i.p.) or buspirone[13] (0.1 mg/kg, i.p.) administration.

Light/Dark Exploration Test in Mice

The apparatus consisted of an open-topped rectangular box, divided into a small area ($27 \times 18 \times 45$ cm high) and a large area ($27 \times 27 \times 45$ cm high) with an open door (7×7 cm) located in the center of the partition at floor level. The small area was painted black and illuminated under a dim red light (40 W). The large compartment was painted white and brightly illuminated with a 100 W light source. Bulbs were located 37 cm above the box. The floor of each compartment was marked into 9-cm squares. Each mouse was placed individually in the centre of the white area. Behavior was recorded over a 5-min period and the time spent in the white or black compartment after drug treatment was calculated.[14] The animals were introduced into the apparatus 40 min after Km administration or 30 min after diazepam (0.1 and 0.5 mg/kg, i.p.) or buspirone (0.1 mg/kg, i.p.) administration.

Elevated Plus-Maze Test in Mice

The elevated plus-maze was made of wood and consisted of two open arms, 30×5 cm and two closed arms, $30 \times 5 \times 15$ cm in height, with an open square of 5×5 cm in the center of the "plus" sign. The maze was elevated to a height of 50 cm. At the beginning of a trial the mice was placed in the centre of the maze facing one of the open arms and allowed to explore the maze for 5 min. The mice were observed via a video camera mounted directly above the center of the maze. The camera was fitted with a polarizing lens and was connected to a television monitor and a PC computer via a video-one Mex (Columbus, USA). The computer software calculated the number of entries and total time spent in each type of arm.[15] The maze was thoroughly wiped clean after each trial. The animals were introduced

into the apparatus 40 min after Km administration or 30 min after diazepam (0.1 and 0.5 mg/kg, i.p.) or buspirone (0.1 mg/kg, i.p.) administration.

Flinch-Jump Test

The threshold of flinch, jump or vocalization produced by electric shock was measured. Each rat was placed in the dark compartment of an inhibitory avoidance task apparatus and the shock intensity was manually raised stepwise from 0.5 to 1.0 mA in increments of 0.1 mA until either a flinch, jump or vocalization was observed. Duration of the shock was 2 s and the intershock interval was 15 s. The point at which the rat exhibited vocalization was recorded.[16] The rats were given 0.03, 0.3 and 1.0 g/kg doses of Km.

Drugs

Buspirone hydrochloride and diazepam were purchased from Sigma Co.. Both drugs were dissolved in 0.9 % saline and injected i.p. in a concentration of 1.0 mg/mL. Control animals received the appropriate volume of vehicle.

Statistical Analysis

All data obtained during the two-way avoidance, light/dark exploration test and elevated plus-maze tests were expressed in terms of medians and interquartile ranges and further analyzed by Kruskal-Wallis non-parametric one-way analysis of variance, followed by Mann-Whitney's U-test. In addition, motor activity and flinch jump test data was analyzed using a one-way analysis of variance, followed by Duncan's multiple range test. The criterion for statistical significance was $P \leq 0.05$ in all statistical evaluations.

RESULTS

Effect of Km on Two-Way Avoidance

Km at 0.3 and 1.0 g/kg decreased the response counts to unconditioned stimulus and intertrial crossing (Fig. 1 and Fig. 2), but did not decrease the response to conditioned stimulus in two-way active avoidance tasks (Fig. 3). Diazepam and buspirone (0.1 mg/kg) also decreased the response counts to unconditioned stimulus and intertrial crossing without decreasing the response to conditioned stimulus.

Effect of Km on Light/Dark Exploration

As shown in Fig. 4, Km at 0.3 and 1.0 g/kg increased the time spent in the white chamber and decreased the time spent in the black chamber during the black/white exploration test, whereas 0.03 g/kg Km had no effect. Diazepam at 0.5 but not 0.1 mg/kg increased the

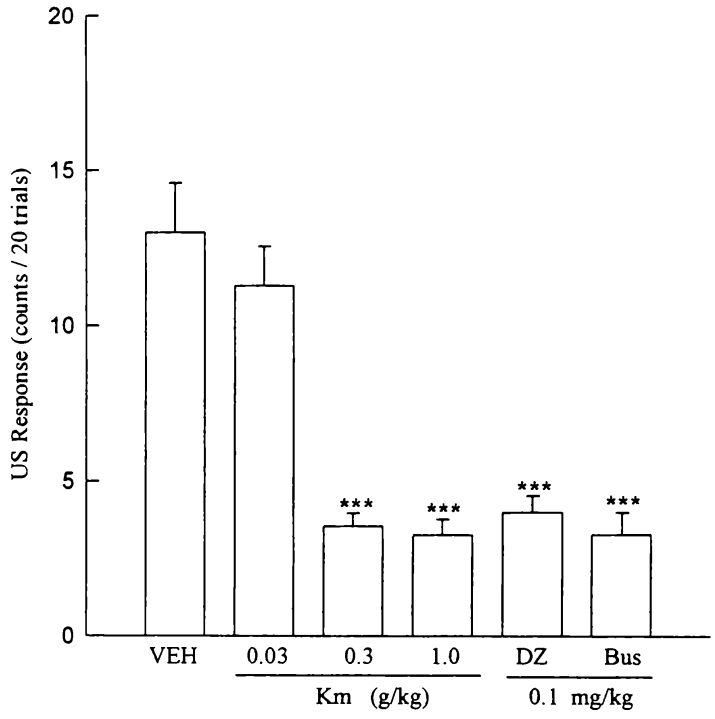

Fig. 1. Effects of Kan-Mai-Ta-Taso-Tang (Km), diazepam (DZ) and buspirone (Bus) on the response to unconditioned stimulus (US) in the two-compartment active avoidance task during a 20-trial test. Scores are means ± SEM. *** $P < 0.001$, significantly different from animals treated with vehicle (VEH). (Mann-Whitney *U* test)

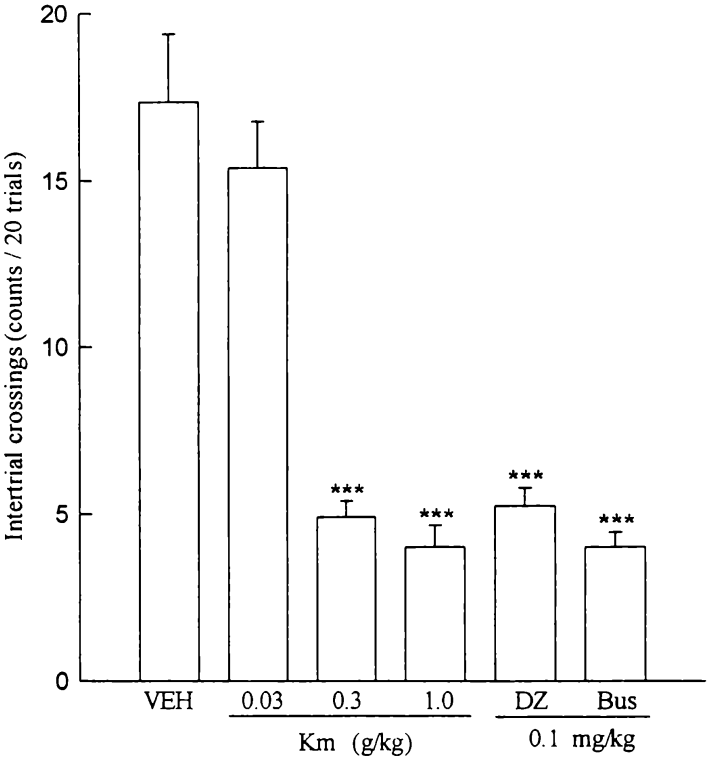

Fig. 2. Effects of Km, DZ and Bus on intertrial crossings in the two-compartment active avoidance task during a 20-trial test. Scores are means ± SEM. *** $P < 0.001$, significantly different from animals treated with vehicle (VEH). (Mann-Whitney *U* test)

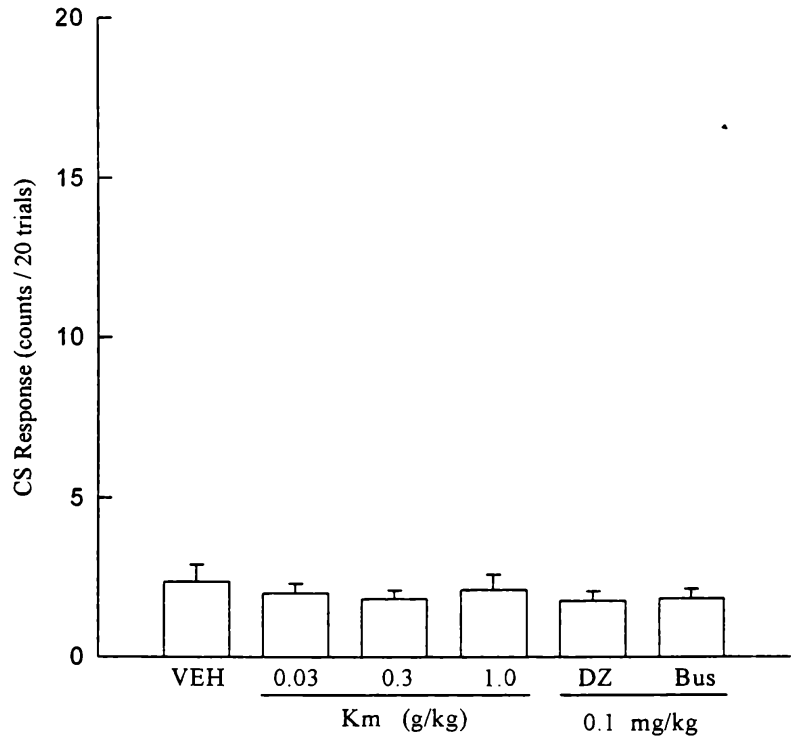

Fig. 3. Effects of Km, DZ and Bus on the response to conditioned stimulus (CS) in the two-compartment active avoidance task during a 20-trial test. Scores are means ± SEM.

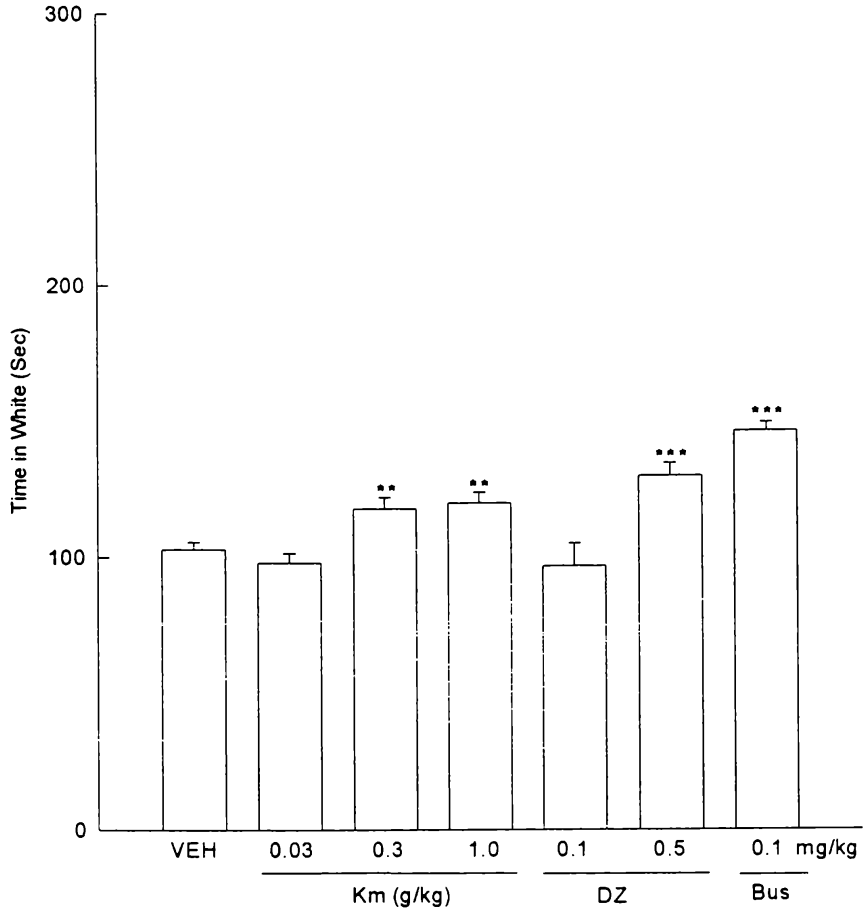

Fig. 4. Effects of Km, DZ and Bus in the black/white exploration during a 5-min test. Time spent in the white chamber. Scores are means ± SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from animals treated with vehicle (VEH). (Mann-Whitney *U* test)

time spent in the white chamber while buspirone at 0.1 mg/kg increased the time spent in the white chamber.

Effect of Km in the Elevated Plus-Maze Test

As shown in Fig. 5, the highest dose of Km (1.0 g/kg) increased the percentage of entries into the open arms of the maze. Moreover, Km at 0.3 and 1.0 g/kg increased the percentage of time spent in the open arms (Fig. 7). Buspirone at 0.1 mg/kg increased the percentage of time spent in the open arms, but did not alter the percentage of entries into the open and closed arms (Fig. 6). Diazepam at 0.5 mg/kg increased the percentage of the entries and time spent in the open arms.

Effect of Km on Pain Threshold

As shown in Table 1, the flinch and vocalization thresholds of rats treated with Km at 0.03, 0.3 and 1.0 mg/kg were not different from vehicle-treated rats.

DISCUSSION

It is well known that many drugs such as benzodiazepines, phenobarbital, valproate and ethanol possess anxiolytic and sedative properties.[10] Since a recent report indicated that Km possessed sedative properties,[9] possible anxiolytic effects of Km were investi-

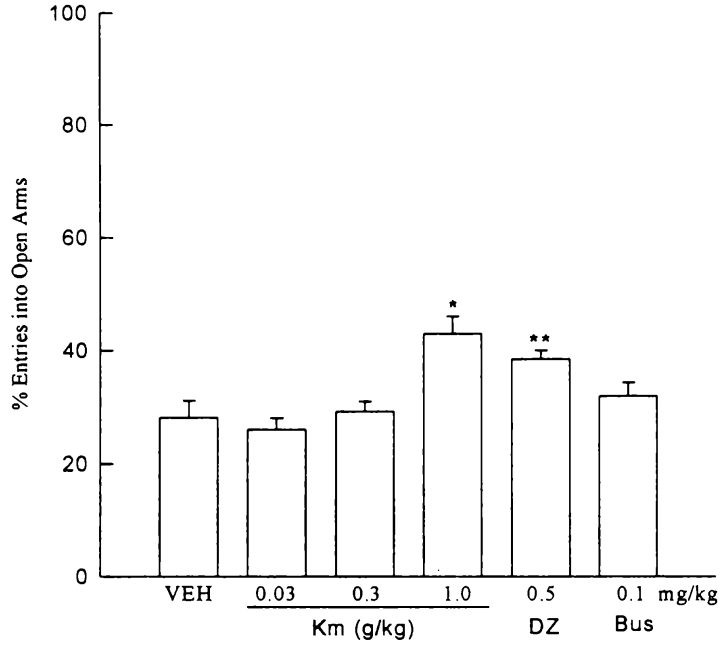

Fig. 5. Effects of Km, DZ and Bus on the percentage of entries made into the open arms of a plus-maze during a 5-min test. Scores are means ± SEM. * $P < 0.05$, ** $P < 0.01$, significantly different from animals treated with vehicle (VEH).

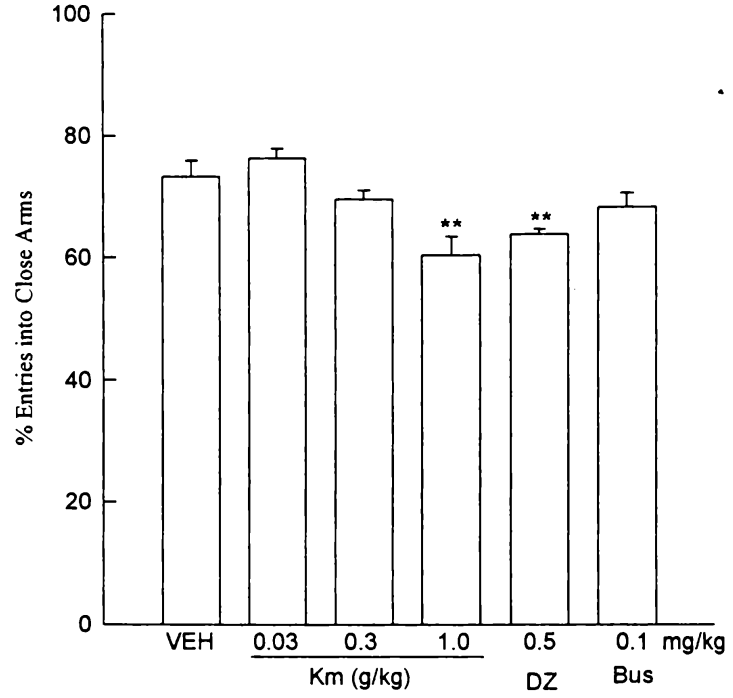

Fig. 6. Effects of Km, DZ and Bus on the percentage of entries made into the closed arms of a plus-maze during a 5-min test. Scores are means ± SEM. ** $P < 0.01$, significantly different from animals treated with vehicle (VEH).

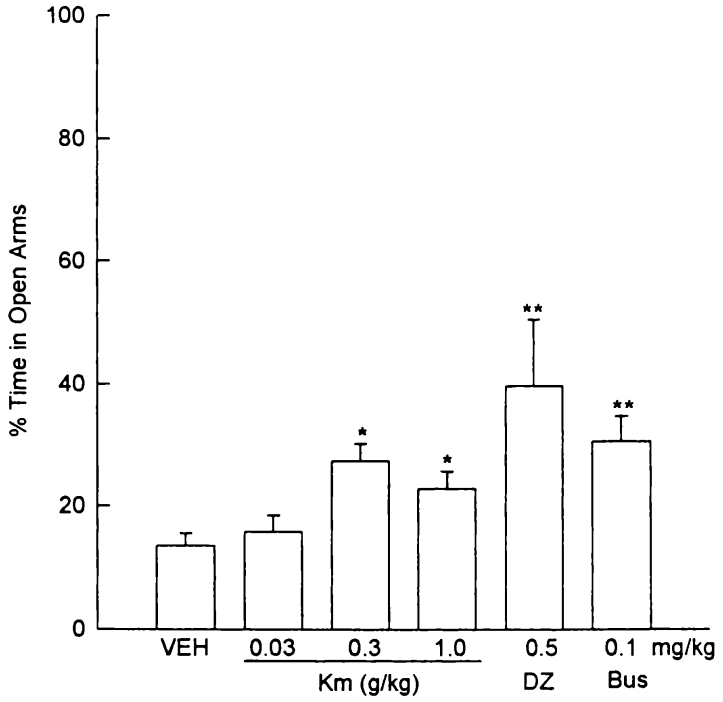

Fig. 7. Effects of Km, DZ and Bus on the percentage of time spent in the open arms of a plus-maze during a 5-min test. Scores are means ± SEM. * $P < 0.05$, ** $P < 0.01$, significantly different from animals treated with vehicle (VEH).

Table 1. Effects of Km on the Response to Electric Foot-Shock in Rats

Drug	Dose (g/kg)	Threshold (mA)	
		Flinch	Jump / Vocalization
VEH		0.74 ± 0.01	0.91 ± 0.03
Km	0.03	0.72 ± 0.03	0.89 ± 0.02
Km	0.3	0.69 ± 0.02	0.90 ± 0.03
Km	1.0	0.74 ± 0.02	0.87 ± 0.01

The results are expressed as mean ± S.E.M for 8 rats each group.

gated. In the present study, we used animal models of anxiety such as the two-way active avoidance test,[11] black/white exploration[14] and the elevated plus-maze[15] to measure anxiolytic effects of Km orally administered at doses which did not produce sedative effects.

In the two-way active avoidance model of anxiety, Km (0.3 and 1.0 g/kg) decreased the response counts to unconditioned stimulus and intertrial crossing but did not alter the response to conditioned stimulus. The results of the two positive controls, diazepam and buspirone, were similar to early reports.[12-13] According to Prunell *et al.*,[11] the results of the first 20 trials mainly involve conflict behavior but not the learning acquisition process. However, the learning acquisition process is mainly involved after more than 20 trials. Our results showing that the vehicle, Km and the two positive controls did not alter the response to conditioned stimulus suggest that the experimental design employing the initial 20 trials primarily involved emotional and conflict behavior and indicate that Km possessed anxiolytic properties in the two-way active avoidance model of anxiety. On the other hand, drug affects on sensory, motivational or motor systems can affect the avoidance response. The vocalization threshold of rats treated with Km in the two-way active avoidance task, however, was grossly normal indicating that the anxiolytic effects of Km were not related to analgesic effects.

In the black/white exploration test, Km (0.3 and 1.0 g/kg) increased the time spent in the white chamber. The results with buspirone were similar to an earlier report.[13] Diazepam required 0.5 mg/kg to increase the time spent in the white chamber, similar to the results reported by Wolfman *et al.*.[17] The results observed in the two animal models of anxiety suggested that Km induces the anxiolytic effects in rodents.

Finally, Km at 0.3 g/kg increased the time spent in the open arms while 1.0 g/kg Km also increased the percentage of entries into the open arms. Buspirone also increased the time spent in the open arm, but did not alter the percentage of entries. Diazepam increased both the percentages of the entries and the time spent in the open arms. These results show that the anxiolytic effects of Km at 0.3 g/kg were similar to buspirone while Km at 1.0 g/kg produced anxiolytic effects more similar to those of diazepam.

Animal models have typically been pharmacologically validated based of their relative sensitivity to acutely administered benzodiazepine- or barbiturate-type anxiolytic drugs.[10] But these anxiety models are relatively insensitive to antidepressants such as tricyclics and monoamine oxidase inhibitor (MAOI). In the plus-maze, rats normally avoid the two elevated open areas of the maze, choosing instead the two enclosed arms. Benzodiazepine-type anxiolytics decrease the avoidance of rats to the open arms, whereas anxiogenic drugs increase the avoidance.[18] However, antidepressants have no reliable effects on open-arm exploration by rats.[19-21] Km at 0.3 g/kg and buspirone did not affect the rats' avoidance of the open arms, although they demonstrated anxiolytic activity in the other two animal models of anxiety- the two- way active avoidance test and black/white exploration. According to early reports of antidepressants in animal models,[21] the anxiolytic effects of Km at 0.3 g/kg were similar to those produced by 5-HT_{1A}-type anxiolytics such as buspirone or ipsapirone, which mainly act via the monoaminergic system.[21,22] Km at 1.0 g/kg and diazepam increased the percentage of entries into the open arms. Paslawski et al.[23] pointed out that high dose of antidepressant drug phenelzine produced anti-anxiety effects mainly by increasing the brain levels of GABA whereas anti-anxiety effects in the elevated plus-maze were not produced at low doses in which only the brain levels of monoamines were changed. According to our results and early reports of benzodiazepine-type anxiolytics[18] and antidepressant drugs,[23] the anxiolytic effects of Km at 1.0 g/kg were similar to those of diazepam.

Therefore, Km at 0.3 and 1.0 g/kg possessed anxiolytic properties in animal models of anxiety, and these anxiolytic effects were not related to analgesic effects. The studied dosage of Km was higher than most traditional clinical applications because a higher dose for acute administration was required to produce the effect. Daily administration is widely used in traditional Chinese medication, so the anxiolytic effects of Km at lower dosage (0.03 or 0.3 g/kg) for long-term administration should be investigated. We will also examine the effects of combinations of Km with monoaminergic drugs on brain monoamines to detemmine the mechanism of action.

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